organs that are widely distributed in the other classes of the Aschelminthes (*11*), but as long as no electron-microscopic studies of these organs in the other groups are available all comparisons should be considered premature. Nevertheless, the demonstration of true cilia in nematodes lessens the gap that exists between the nematodes and the remainder of the aschelminths.

D. R. ROGGEN
D. J. RASKI
N. O. JONES

Department of Nematology, University of California, Davis

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Synthesis of Nonribosomal RNA by Lymphocytes: A Response to Phytohemagglutinin Treatment

Abstract. *When lymphocytes are stimulated to enlarge and divide by treatment with phytohemagglutinin, most of the rapidly synthesized RNA is nonribosomal. The phenomenon is a response by lymphocytes to stimulation by phytohemagglutinin, rather than a general concomitant of lymphocyte growth.*

When phytohemagglutinin (PHA), extracted from kidney beans (*1*), is added to suspensions of human peripheral blood lymphocytes, there occurs a prompt increase in the rate of RNA synthesis (*2*). A period of growth ensues, and during the succeeding 60 to 72 hours most of the cells undergo progressive enlargement and mitosis (*3, 4*). We have reported (*5*) that under these conditions most of the rapidly synthesized RNA in lymphocytes appears to be nonribosomal. This material sediments in a sucrose gradient as a broad band with sedimentation constants from 6*S* to more than 20*S*. Our findings differed from reported observations with other cultured cells, where the largest fraction of rapidly synthesized RNA is a 45*S* precursor which is subsequently converted to ribosomal RNA (*6, 7*). We also observed this fraction in PHA-stimulated lymphocytes (*5*), but it constituted only a minor portion of the pulse-labeling RNA. Our previous studies did not permit us to distinguish between two possible interpretations of our findings: (i) whether the type of RNA metabolic pattern observed was an obligatory property of growing lymphocytes or (ii) whether PHA specifically stimulated a high rate of production of nonribosomal RNA in lymphocytes.

To clarify this problem, we compared the RNA synthetic activity of PHA-stimulated lymphocytes with that of cells exposed to streptolysin O, another substance which provides a mitogenic stimulus to lymphocytes (*4, 8*). Streptolysin O is effective only in lymphocytes from subjects previously exposed to hemolytic streptococci, and it apparently acts as a specific antigenic stimulus to sensitized cells (*4, 8*). Phytohemagglutinin requires no prior exposure, and stimulates a much larger proportion of the lymphocyte population.

We found that stimulation of lymphocytes by a specific antigen resulted in the production of ribosomal precursor as the largest fraction of rapidly synthesized RNA, but confirmed that the major component of pulse-labeling RNA produced by PHA-treated cells is nonribosomal. The pattern of RNA metabolism of lymphocytes treated with growth stimulating agents, therefore, is not fixed, but depends on the stimulating agent used.

Human peripheral lymphocyte suspensions were prepared (*2, 5*) in Eagle's minimum essential medium (spinner modification) containing 10 percent autologous plasma and antibiotics. Phytohemagglutinin P and streptolysin O obtained from Difco were added as growth stimulants to appropriate cultures. Newly synthesized RNA was labeled with uridine-H^3, and whole-cell RNA was extracted. The technique for RNA extraction, based on that of Scherrer and Darnell (*6*), has been modified from that previously used (*2, 5*) to provide increased yields and to minimize RNA degradation. Labeled cells were chilled and washed in Earle's salt solution. All steps were carried out at 0° to 4°C, except as noted. The cells were suspended in .01*M* acetate buffer, *p*H 5.1, containing 0.1*M* NaCl, 0.001*M* $MgCl_2$, and 1 percent bentonite and then made 0.34 percent to sodium dodecyl sulfate. The suspension was treated with high-frequency sound for 3 to 5 seconds and then shaken manually for 1 minute. An equal volume of redistilled, buffer-saturated phenol, containing 0.1 percent of 8-hydroxyquinoline, was added; the emulsion was then mixed at 60°C for 5 minutes and rapidly chilled. The emulsion was broken by centrifugation and the aqueous phase was reextracted with hot phenol for 3 minutes. To the final aqueous phase,

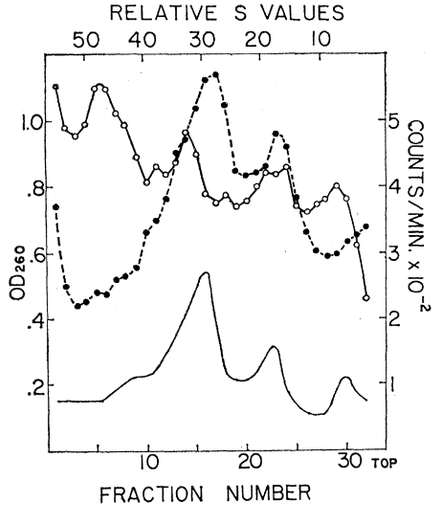

Fig. 1. Effect of treatment with actinomycin D on rapidly synthesized RNA from lymphocytes stimulated with streptolysin O. Parallel cultures, 5 × 10^7 lymphocytes each, 2 × 10^6 cells/ml, were incubated for 7 days with streptolysin O (4 ml of standard streptolysin O reagent per culture). Both cultures were incubated with uridine-H^3 (10 μ/ml, 4 to 7 c/mmole) for 30 minutes. One culture was then harvested ("pulse"), and actinomycin D (10 μg/ml) and unlabeled uridine (2 mg) were added to the other. After 1 hour of further incubation this culture was harvested ("chase"). RNA was extracted and sedimented in a sucrose gradient (5 to 20 percent) at 25,000 rev/min for 11 hours in a Spinco SW25 rotor. The gradient was pumped through a recording spectrophotometer to determine ultraviolet absorbancy (OD_{260}), and 30 to 35 serial fractions were collected and analyzed for radioactivity in a liquid-scintillation counter. ———, OD_{260}; ○———○, count/min for pulse; ●———●, count/min for chase. Relative sedimentation constants were calculated (*13*).

unlabeled rabbit or rat liver RNA was added as carrier, and the RNA was precipitated with two volumes of alcohol at −20°C. The precipitate was dissolved in acetate buffer, purified by passage through Sephadex G-25, and precipitated again with alcohol. The precipitated material was analyzed by sucrose density-gradient ultracentrifugation (*2, 5*). For analysis of the base ratios in the RNA, P^{32}-labeled samples from sucrose gradients were collected in an ice bath. Portions of each sample were dried and subjected to acid precipitation on filter paper for assay of radioactivity (*9*); the remainder of the fractions were combined as pools representing different regions of the gradient. Yeast RNA was added as carrier, and RNA was precipitated at 4°C from the pools with 2 percent perchloric acid and hydrolyzed for 18 hours with 0.3*N* KOH at 37°C. Separation of ribonucleotides was performed by thin-layer electrophoresis (*10*) with pyridine-acetate buffer (*11*). Nucleotide spots were located under ultraviolet light and were removed from the thin-layer plates with a razor, after being sprayed with Neatan (*12*). Radioactivity of the cellulose spots was assayed in a liquid-scintillation counter.

When lymphocytes from a susceptible subject were incubated with streptolysin O for 7 days (a time when antigen-stimulated cell enlargement and mitotic activity were maximum), pulse-labeling RNA sedimented in polydisperse fashion (Fig. 1). However, the largest accumulation of rapidly synthesized RNA sedimented at 45 to 50*S* (*13*). We have obtained similar results with tuberculin as a specific antigen in place of streptolysin O. This finding is similar to that reported for rapidly synthesized RNA produced in vivo in antigen-stimulated lymphoid tissues (spleen and lymph node) of the rat (*14*), and also resembles observations on other cell culture systems (*6, 7*). When pulse-labeled cells were "chased" by subsequent exposure to actinomycin D and unlabeled uridine for 1 hour, most of the label incorporated during the pulse period (usually > 60 percent) remained in macromolecular form and sedimented with ribosomal RNA, as seen by comparison with the ultraviolet-absorbancy profile (Fig. 1). The prominent 45 to 50*S* peak of pulse-labeled RNA was clearly absent after the chase.

Our results are consistent with the widely held concept that the 45*S* material is a precursor which is subsequently converted to ribosomal RNA (*6, 7*). Apparently this ribosomal precursor was extracted by our method, and, under the conditions used, actinomycin D neither caused its degradation nor prevented its conversion to 18*S* and 28*S* moieties. Thus a large portion of the rapidly synthesized RNA in antigen-stimulated lymphocytes appears to be ribosomal precursor, although it is evident that nonribosomal RNA is also actively produced.

Table 1. Nucleotide composition of rapidly synthesized RNA from lymphocytes incubated with PHA for 60 hours, compared with that of ribosomal RNA. Rapidly synthesized RNA was extracted after a 30-minute incubation with $H_3P^{32}O_4$ (3 mc/ml) and analyzed for base composition. Peak of pulse-labeling RNA in this representative experiment was in the 15 to 24*S* region. Ribosomal RNA was labeled by a 2-hour incubation of PHA-stimulated lymphocytes with $H_3P^{32}O_4$, followed by a 3-hour incubation with excess nonradioactive phosphate, and finally by a 1-hour "chase" treatment with actinomycin D. The remaining radioactive RNA sedimented entirely with ribosomal RNA.

Moles per 100 moles				G+C/ A+U
U	G	A	C	
7 to 15S RNA, pulse-labeled				
27.3	28.2	25.7	18.8	0.89
15 to 24S RNA, pulse-labeled				
26.3	25.4	26.6	20.8	0.86
24 to 39S RNA, pulse-labeled				
26.3	26.9	24.4	22.3	0.97
39 to 55S RNA, pulse-labeled				
25.7	29.3	21.5	23.2	1.11
30S ribosomal RNA				
18.9	33.3	20.9	26.9	1.51
18S ribosomal RNA				
21.8	30.6	20.3	27.3	1.38

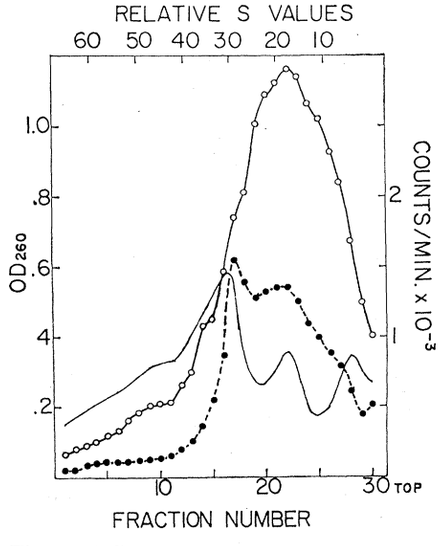

Fig. 2. Effect of treatment with actinomycin D on rapidly synthesized RNA from PHA-stimulated lymphocytes. Details as in Fig. 1, except that cells were incubated for 60 hours with PHA (25 μg/10^6 cells) instead of with streptolysin O. Sedimentation was at 25,000 rev/min for 10.5 hours. ———, OD_{260}; O———O, count/min for pulse; ●———●, count/min for chase. There is a scale difference for count/min between Figs. 1 and 2.

In similar studies of lymphocytes incubated with PHA for 60 hours, pulse-labeled RNA sedimented with a small peak or shoulder at 45 to 50*S* and a larger broad band of material extending from 6*S* to 30*S* (Fig. 2). The location of the peak of this band varied, in different experiments, from near 6*S* to over 20*S*. After the actinomycin chase treatment, a large portion of this material was not recovered (Fig. 2). The sedimentation pattern of the remaining labeled material only roughly approximated that of ribosomal RNA, an indication that only a fraction of the radioactivity remaining after the chase treatment was located in ribosomal RNA (*15*). Thus, a major portion of rapidly-synthesized RNA in the PHA-stimulated cultures was degraded in the presence of actinomycin D, not converted to ribosomal RNA as would be expected of ribosomal precursor. Since only a portion of the remaining labeled RNA appears to be ribosomal, it may be concluded that a quite small segment of the original pulse-labeled RNA was ribosomal precursor.

Analysis of RNA base composition (Table 1) revealed a high U-A (*16*) and relatively low G-C content for the peak region of pulse-labeling RNA in PHA-stimulated cultures (Table 1 : 15 to 24*S*). This differs from the high G-C and low A-U content found for ribosomal RNA (Table 1: 18*S* and 30*S*), which result agrees with other studies of the nucleotide composition of mammalian ribosomal RNA (*6, 17*). The pulse-labeling RNA with base composition most closely resembling ribosomal RNA was found in the 39 to 55*S* region of the gradient, which contains the ribosomal precursor.

The largest fraction of rapidly synthesized RNA in phytohemagglutinin-stimulated lymphocytes is seen to be nonribosomal by three criteria: (i) Its sedimentation profile in sucrose gradients is unlike ribosomal precursor or ribosomal RNA. (ii) It is relatively unstable to chasing with actinomycin D. (iii) Its nucleotide composition is unlike that of ribosomal RNA. We have shown that when lymphocytes enlarge and divide in response to a specific

antigen they exhibit a pattern of RNA metabolism similar to that of other cell culture systems (*6, 7*) in which the largest single accumulation of pulse-labeled RNA is precursor to ribosomal RNA. The unusually rapid rate of synthesis of nonribosomal RNA seen after PHA stimulation is, therefore, not obligatory for growing lymphocytes, but must be a response to treatment with PHA.

We also have evidence that the continuous presence of PHA is required for this phenomenon. When PHA-stimulated lymphocytes are washed, the RNA metabolic pattern appears to revert to the predominant synthesis of 45 to 50*S* material, although cell growth does not diminish. This alteration sometimes occurs without washing, after 60 to 70 hours of incubation with PHA, especially if low starting concentrations are used. Addition of excess PHA restores the major production of 6 to 30*S* RNA, again demonstrating that PHA treatment is responsible for this pattern of RNA synthesis.

The precise nature and function of the abundant nonribosomal RNA produced by lymphocytes in the presence of PHA are unknown, but an obvious hypothesis is that it is messenger RNA being produced in large quantity, perhaps as a result of the abrogation of normal regulatory processes by PHA.

HERBERT L. COOPER
National Institute of Dental Research, Bethesda 14, Maryland 20014
ARNOLD D. RUBIN
Blood Research Laboratory, New England Medical Center, Boston, Massachusetts

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15. In previous studies (*5*) the sedimentation pattern of labeled RNA remaining after the chase in PHA-stimulated cells showed good correspondence with the absorbancy profile. The difference in the present work is due to the more complete extraction of nonribosomal RNA achieved by our current methods.
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3 March 1966

Mitotic Waves in Laticifers of Euphorbia marginata

Abstract. *A successive pattern of nuclear divisions that result in mitotic waves has been observed within the coenocytic nonarticulated laticifers of embryos of* Euphorbia marginata *Pursh. These waves originate independently in the cotyledonary or hypocotyl portion of the laticifer and exhibit uni- or bidirectional movement at variable velocities. Individual nuclei or groups of neighoring nuclei in a laticifer were observed in a sequence of mitotic stages ranging from prophase to telophase; division activity varied with individual laticifers in an embryo. Two mitotic patterns were apparent in the embryo: a random pattern associated with various cells in the meristematic area, and a successive pattern restricted to the laticifer. A substance, synthesized by and restricted to the laticifer, may be associated with this mitotic pattern.*

Mitotic activity within organisms can be broadly categorized into random, synchronous, and successive patterns of division. Random distribution is the most common and characterizes mitotic activity in vegetative apices (*1, 2*). The simultaneous division pattern is most frequently observed in various megagametophytes (*3*), endosperms (*4*), proembryos (*5*), antheridia (*6*), sporangia (*7*) of plants, and eggs of animals (*8*). Frequency of the successive pattern of mitotic division is comparatively low and has been reported in endosperm (*9*), laticifers (*10*), and in some animal eggs where an initial synchronous pattern is followed by successive mitotic waves (*8*).

Successive division that results in a mitotic wave occurs in the nonarticulated laticifer, a specialized coenocytic cell in *Euphorbia marginata* Pursh. A limited number of laticifers, a cell type restricted to only a few families of flowering plants, originates during the early heart stage in embryo development. The laticiferous cell branches and it also becomes coenocytic by repeated divisions of its nucleus. In addition, some branches of the laticifer permeate downward into the hypocotyl and penetrate very close to the root meristem, while others extend into the developing epicotyl. The hypocotyl is an excellent location for study of the division pattern in the laticifer because a considerable number of nuclei occur in a row within a relatively short length of the cell.

Observations were made from paraffin-embedded histological sections of immature embryos of *E. marginata* in various stages of development.

Division sequence for a row of nuclei in the laticifer consistently follows the successive pattern of mitosis. Mitotic activity is not randomly distributed along a laticifer.

Two sites of origin of mitotic waves, one in the middle of the cotyledon and the other in the upper half of the hypocotyl, have been observed. The mitotic wave that originates in the cotyledon exhibits a unidirectional movement toward the cotyledonary node, while in the hypocotyl it may display either unidirectional or bidirectional flow.

Only one uni- or bidirectional wave was observed in any one laticifer. From the point of origin, a retrogressive sequence of divisional stages from telophase to prophase was observed in both directions along the laticifer, or in only one direction (Fig. 1). This successive pattern of mitotic activity suggests that division is controlled by a substance diffusing along the axis of the laticifer.

The mitotic waves in the cotyledonary and hypocotyl branches of the laticifer are out of phase. When nuclear divisions were observed in the cotyledonary portion of the laticifer, none were evident in the hypocotyl axis of the cell.

The length of mitotic waves varied both in the same laticifer and in different ones. A wave consisted of as few as 4 (Fig. 1) or as many as 28 nuclei, representing all stages of mitosis. The shorter wave usually occurred in the upper half of the cotyledon or in the cotyledonary node. Temporally, metaphases and telophases were the